

**Amendments to the Claims**

This listing of claims will replace all prior versions, and listings, of claims in the application.

**Listing of Claims:**

1. (currently amended) A method of isolating adult cardiac cells comprising,
  - (a) obtaining a tissue sample from a subject,
  - (b) successively exposing the tissue to a first solution with decreasing amounts of  $\text{CaCl}_2$  decreasing from about 1-2  $\mu\text{M}$ , comprising  $\text{NaCl}$ , HEPES,  $\text{MgCl}_2$ ,  $\text{KCl}$ , and sugar at a pH of approximately 7.4,
  - (c) disassociating the tissue with an enzyme solution,
  - (d) repeatedly resuspending the disassociated tissue into a second solution with increasing amounts of  $\text{CaCl}_2$  increasing from about 1-2  $\mu\text{M}$ , comprising modified Earle's modified salt, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, fetal bovine serum, and an antibiotic, and a fatty acid, at a pH of approximately 7.4 to obtain isolated cells.
2. (currently amended) The method of claim 1, further comprising the step of resuspending the isolated cells approximately every 24 hours in a solution comprising modified Earle's modified salt, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, fetal bovine serum, an antibiotic, a fatty acid, and  $\text{CaCl}_2$  at a pH of approximately 7.4.
3. (original) The method of claim 1, further comprising the step of incubating the isolated cells in a mixture of carbon dioxide and air.
4. (original) The method of claim 3, wherein the isolated cells are incubated at approximately 37°C.
5. (original) The method of claim 1 wherein, the first solution is exposed to the tissue at approximately 37°C and at approximately 4 ml/min for 3 minutes.

6. (original) The method of claim 1 wherein the concentration of CaCl<sub>2</sub> in the first solution decreases.

7. (original) The method of claim 1 wherein the first solution comprises approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM MgCl<sub>2</sub>, approximately 5.4 mM KCl, and approximately 10 mM D-glucose.

8. (original) The method of claim 1 wherein the enzyme solution comprises a digestive enzyme.

9. (original) The method of claim 8, wherein the digestive enzyme is a protease or a collagenase.

10. (original) The method of claim 1 wherein the concentration of CaCl<sub>2</sub> in the second solution increases.

11. (original) The method of claim 1 wherein the enzyme solution comprises approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM MgCl<sub>2</sub>, approximately 5.4 mM KCl, and approximately 10 mM D-glucose.

12. (currently amended) The method of claim 1 wherein the second solution comprises modified Earle's modified salt, L-glutamine, sodium bicarbonate at approximately 1250mg/l, sodium pentothenate, creatine at approximately 328 mg/500ml, taurine at approximately 312mg/500ml, Ascorbic acid at approximately 8.8 mg, HEPES at approximately 2.383g/500ml, ~~fetal bovine serum at approximately 10% v/v, and an antibiotic at approximately 5% v/v, a fatty acid at approximately 1  $\mu$ M~~ at a pH of approximately 7.4.

13. (currently amended) A method of isolating adult cardiac cells comprising,

(a) obtaining a tissue sample from a subject,

(b) successively exposing at approximately 37°C the tissue to a first solution with ~~decreasing amounts of CaCl<sub>2</sub> decreasing from about 1-2  $\mu$ M~~, comprising approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM MgCl<sub>2</sub>, approximately 5.4 mM KCl, and approximately 10 mM sugar at a pH of approximately 7.4,

(c) disassociating the tissue with an enzyme solution for approximately 8 minutes comprising approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM

MgCl<sub>2</sub>, approximately 5.4 mM KCl, and approximately 10 mM sugar, to form disassociated cells,

(d) repeatedly resuspending the disassociated cells into a second solution with increasing amounts of CaCl<sub>2</sub> increasing to about 1-2 $\mu$ M, comprising modified Earle's modified salt, L-glutamine, sodium bicarbonate at approximately 1250mg/l, sodium pentothenate, creatine at approximately 328 mg/500ml, taurine at approximately 312mg/500ml, ascorbic acid at approximately 8.8 mg, HEPES at approximately 2.383g/500ml, fetal bovine serum at approximately 10% v/v, and an antibiotic at approximately 5% v/v, and a fatty acid at approximately 1  $\mu$ M at a pH of approximately 7.4 to form a solution of isolated cells,

(e) incubating the isolated cells in a mixture of carbon dioxide and air at approximately 37°C, and

(f) re-suspending the isolated cells approximately every 24 hours in a solution comprising modified Earle's modified salt, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, fetal bovine serum, an antibiotic, a fatty acid, and CaCl<sub>2</sub> at a pH of approximately 7.4 to obtain isolated cells.

14. (withdrawn) A method of cultivating isolated cells comprising, resuspending the isolated cells approximately every 24 hours in a solution comprising Earle's modified salt, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, fetal bovine serum, an antibiotic, a fatty acid, and CaCl<sub>2</sub> at a pH of approximately 7.4.

15. (withdrawn) The method of claim 14 wherein the solution comprises sodium bicarbonate at approximately 1250mg/l, creatine at approximately 328 mg/500ml, taurine at approximately 312 mg/500ml, ascorbic acid at approximately 8.8 mg/500 ml, HEPES at approximately 2.383 g/500ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, and a fatty acid at approximately 1  $\mu$ M, and approximately 1mM CaCl<sub>2</sub>.

16. (withdrawn) A cell culture media for cells comprising Earle's modified salt, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, fetal bovine serum, an antibiotic, a fatty acid, and CaCl<sub>2</sub> at a pH of approximately 7.4.

17. (withdrawn) The cell culture media of claim 16 wherein the media comprises sodium bicarbonate at approximately 1250mg/l, creatine at approximately 328 mg/500ml, taurine at approximately 312 mg/500ml, ascorbic acid at approximately 8.8 mg/500 ml, HEPES at

approximately 2.383 g/500ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, a fatty acid at approximately 1  $\mu$ M, and approximately 1mM CaCl<sub>2</sub>.

18. (withdrawn) A method of isolating cells comprising,
  - (a) obtaining a tissue sample comprising cells from a subject ;
  - (b) chopping the tissue;
  - (c) incubating the tissue in a first solution comprising calcium, salts, magnesium sulfate, pyruvate, glucose, taurine, HEPES, and nitrilotriacetic acid;
  - (d) incubating the tissue in a second solution comprising calcium, salts, magnesium sulfate, pyruvate, glucose, taurine, HEPES, and a digestive enzyme;
  - (e) incubating the tissue in a third solution comprising calcium, salts, magnesium sulfate, pyruvate, glucose, taurine, HEPES, and a digestive enzyme; and
  - (f) centrifuging the tissue to obtain isolated cells .
19. (withdrawn) The method of claim 18, further comprising the step of resuspending the isolated cells in a culture media comprising medium M199, BSA, ascorbic acid, taurine, carnitine, creatinine, insulin, and an antibiotic .
20. (withdrawn) The method of claim 19, wherein the culture media further comprises a fatty acid or magnesium.
21. (withdrawn) The method of claim 18, wherein the first solution comprises approximately 1-2  $\mu$ M CaCl<sub>2</sub>, approximately 120mM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM MgSO<sub>4</sub>, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and approximately 5 mM nitrilotriacetic acid, at a pH of approximately 6.96.
22. (withdrawn) The method of claim 18, wherein the second solution comprises approximately 1-2  $\mu$ M CaCl<sub>2</sub>, approximately 30  $\mu$ M NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM MgSO<sub>4</sub>, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme.
23. (withdrawn) The method of claim 18, wherein the third solution comprises approximately 1-2  $\mu$ M CaCl<sub>2</sub>, approximately 30  $\mu$ M NaCl, approximately 5.4 mM KCl 5.4,

approximately 5 mM MgSO<sub>4</sub>, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme.

24. (withdrawn) A method of isolating cells comprising,  
(a) obtaining a tissue sample comprising cells from a subject ;  
(b) chopping the tissue;  
(c) incubating the tissue in a first solution comprising approximately 1-2  $\mu$ M CaCl<sub>2</sub>, approximately 120mM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM MgSO<sub>4</sub>, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and approximately 5 mM nitrilotriacetic acid, at a pH of approximately 6.96;  
(d) shaking the tissue at approximately 37°C for approximately 12 minutes;  
(e) bubbling approximately 100% O<sub>2</sub> through the solution;  
(f) incubating the tissue in a second solution comprising approximately 1-2  $\mu$ M CaCl<sub>2</sub>, approximately 30  $\mu$ M NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM MgSO<sub>4</sub>, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme;  
(g) incubating the solution in a third solution comprising third solution comprises approximately 1-2  $\mu$ M CaCl<sub>2</sub>, approximately 30  $\mu$ M NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM MgSO<sub>4</sub>, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme; and  
(h) centrifuging the tissue to obtain isolated cells.

25. (withdrawn) A method of isolating and cultivating human myocardial cells comprising,  
(a) obtaining a tissue sample comprising myocardial cells from a human subject;  
(b) chopping the tissue;  
(c) incubating the tissue in a first solution comprising approximately 1-2  $\mu$ M calcium, approximately 120mM NaCl, approximately 5.4 mM KCl, approximately 5 mM MgSO<sub>4</sub>, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and approximately 5 mM nitrilotriacetic acid, at a pH of approximately 6.96;  
(d) shaking the tissue at approximately 37°C for approximately 12 minutes;  
(e) bubbling approximately 100% O<sub>2</sub> through the solution;

(f) incubating the tissue in a second solution comprising approximately 1-2  $\mu$ M, approximately 30  $\mu$ M NaCl, approximately 5.4 mM KCl, approximately 5 mM MgSO<sub>4</sub>, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme;

(g) incubating the solution in a third solution comprising third solution comprises approximately 1-2  $\mu$ M, approximately 30  $\mu$ M NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM MgSO<sub>4</sub>, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 400U/ml of a digestive enzyme;

(h) centrifuging the tissue to obtain isolated cells;

(i) repeatedly resuspending the disassociated cells into a second solution which comprises increasing amounts of CaCl<sub>2</sub>, Earle's modified salt, L-glutamine, sodium bicarbonate at approximately 1250mg/l, sodium pentothenate, creatine at approximately 328 mg/500ml, taurine at approximately 312mg/500ml, ascorbic acid at approximately 8.8 mg, HEPES at approximately 2.383g/500ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, and a fatty acid at approximately 1  $\mu$ M at a pH of approximately 7.4 to form a solution of isolated cells; and

(j) incubating the isolated cells in a mixture of carbon dioxide and air at approximately 37°C.

26. (withdrawn) A method of isolating and cultivating rodent myocardial cells comprising,

(a) removing the heart of a rodent;

(b) perfusing the heart with low calcium Tyrode's solution for approximately 3 minutes;

(c) perfusing the heart with an enzymatic solution for approximately 8 minutes;

(d) perfusing the heart with a low calcium solution for approximately 3 minutes;

(e) removing the ventricles;

(f) mincing the ventricles to isolate myocardial cells;

(g) mixing the cells in a low calcium solution;

(h) resuspending the cells in a solution comprising increasing concentrations of calcium; and

(i) resuspending the cells in culture media solution.